

motion. Here we propose a mechanism whereby electric fields emanating from channels in the stator(s) create forces acting on ion binding sites in the rotor, driving it to rotate. The model predicts a scaling law relating torque to ion motive force and number of stators, consistent with experiment. The rotor of F0 drives the gamma-subunit to rotate within the ATP-producing complex (F1), working against an opposing torque that rises and falls periodically with angular position. Drawing an analogy with the washboard potential of the superconducting Josephson junction, we compute ATP production rate vs. ion motive force, finding excellent quantitative agreement with experiment and accounting for the crossover from ATP production to consumption. Plausible mechanisms for reversing the direction of bacterial flagellar rotation become self-evident in the model. (Supported by NIH R21CA133153, TcSUH, Welch (E-1221), and THECB-ARP.)

Platform D: RNA Folding & Ribosome

44-Plat

Laser Assisted Single-molecule Refolding

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In vivo, many RNA molecules can adopt multiple conformations depending on their biological context. For example, an RNA molecule initially in a stable hairpin conformation may later interact with a second RNA molecule, thus triggering a dimerization reaction. This is the case of the HIV Dimerization Initiation Sequence (DIS) and the DsrA RNA in bacteria. It is quite common that the initial interaction between the two RNAs takes place via complementary unpaired regions, forming a so-called kissing complex. However, the exact kinetic mechanism by which the two RNA molecules reach the dimerized state is still not well understood.

To investigate the refolding energy surface of RNA molecules, we have developed new technology based on the combination of single molecule spectroscopy with laser induced temperature jump kinetics, called Laser Assisted Single-molecule Refolding (LASR). LASR enables us to induce folding reactions of otherwise kinetically trapped RNAs at the single molecule level, and to characterize their folding landscape. Single molecule time trajectories show that we can drive the dimerization reaction between two trapped kissing RNA hairpins with LASR and use this data to calculate folding enthalpies and entropies. Our LASR experiments indicate that the RNA kissing complex is a stable intermediate state that facilitates the dimerization reaction.

LASR provides an exciting new approach to study molecular memory effects and kinetically trapped RNAs in general. LASR is readily applicable to study DNA and protein folding as well.

45-Plat

Single Molecule Analysis of Group I Ribozyme Folding Reveals Pronounced Ruggedness Throughout Its Folding Landscape

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It is well established that biological activity of macromolecules is intimately related to their structures. Significant efforts are directed at characterizing macromolecular structures and structure-function relationships. It is less appreciated that because of their complexity macromolecules are likely to fold not into unique structures, but into ensembles of different conformations distinct in their biological activity. Strong evidence for such complex behavior has been recently obtained in several macromolecular systems, mostly by single molecule methods. Understanding biological activity of macromolecules requires detailed characterization of the species that co-exist in the “folded” states, in other words - mapping of their folding energy landscape.

We used single molecule FRET to map folding energy landscape of a catalytic RNA - ribozyme derived from a group I intron. Labeling several different positions within the ribozyme allowed us to probe different parts of the folding landscape: from essentially unfolded to fully folded ribozyme. These experiments revealed broad heterogeneity of folding behaviors of individual molecules. Strikingly, even in conditions in which the ribozyme is fully folded and active, different conformations retain distinct activity. Distinct folding behaviors are “remembered” by individual molecules upon long incubation in conditions in which the ribozyme lacks most of its tertiary structure. This finding suggests that the origins of heterogeneity might appear at the level of secondary structure. Pinning down the structural basis for ruggedness of RNA folding landscapes and understanding its role in biology represents an exciting future challenge.

46-Plat

Identifying Energy Barriers in RNA Folding Through Kinetic Model Enumeration

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Large RNA molecules are known to fold through multiple parallel pathways to achieve their functional conformation. These pathways include stable intermediate structures that we identify using our KinFold algorithm (<http://simtk.org/home/kinfold>) for the analysis of time-resolved hydroxyl radical footprinting experiments. KinFold enumerates kinetic model topologies to determine the structure, lifetimes, and abundance of intermediates along the folding pathways of RNA. We are therefore able to identify the best fitting kinetic model for each folding reaction studied. We used this approach to study the folding of the L-21 T. thermophila group I intron, a catalytic ribozyme, at 6 temperatures ranging from 25° to 51° C. Our analysis of this data reveals that the rates for a majority of the transitions (between intermediate, folded and unfolded states) obey the Arrhenius equation, allowing us to estimate the relative energies of activation for inter conversion between the states of an RNA. Furthermore, we are able to estimate the lifetimes of the different intermediate structures. These results reveal a simple landscape, where folding of the peripheral elements of the RNA create significant barriers to folding. Our approach is unique in that it allows us to simultaneously estimate the activation energies for all transitions in an RNA folding reaction.

47-Plat

Uncovering The Mechanism Used By Drosophila Snf Protein To Specifically Bind Two RNAs

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The Drosophila SNF protein is a natural chimeric protein that evolved from the metazoan U1A and U2B” proteins. In the fly, SNF is found in both the U1 and U2 snRNPs, where it is assumed to bind to the U1 snRNA Stemloop II (the site of U1A binding) and U2 snRNA Stemloop IV (the site of U2B” binding), respectively. Like U1A and U2B” proteins, SNF has two RNA Recognition Motifs (RRM). By analogy to U1A protein, the N-terminal RRM should bind RNA, while the C-terminal RRM does not. Our experiments show that indeed the SNF C-terminal RRM does not bind RNA; NMR data show it to be soluble and stable. The SNF N-terminal RRM alone is not stable, however, and requires the C-terminal domain to maintain a folded form. NMR experiments and ¹⁵N-relaxation data show that the N-terminal RRM undergoes conformational exchange on the chemical shift timescale, while the C-terminal RRM has uniformly high order parameters characteristic of a more rigid protein.

We hypothesize that the increased flexibility of the N-terminal RRM is part of its RNA binding mechanism, since SNF does indeed bind both U1 snRNA Stemloop II and U2 snRNA Stemloop IV. The affinity of SNF for these two RNAs differs by three orders of magnitude, indicating that one is preferentially bound. By comparison, however, the affinities of the human U1A protein for these two RNAs differ by more than 10⁶-fold, indicating that SNF has found a way to bind both RNAs. This hypothesis will be tested by mutation of the N-terminal domain, with subsequent determination of protein structure, dynamics, and RNA binding.

48-Plat

Parallel Pathways in 30S Ribosome Assembly

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The bacterial 30S ribosomal subunit self-assembles in vitro to form an 850kDa RNP. Assembly is generally thought to advance when the core 16S rRNA navigates through many low-energy kinetic traps, guided by the 20 small-subunit proteins that recognize and lock in native RNA tertiary structure. Kinetic analyses of protein binding reveal a highly choreographed, ordered assembly process, consistent with large-scale qualitative observations of ordered protein binding and measurements of thermodynamic cooperativity within synthetic fragments of the 30S. However, the precise order of events in this complex process are not well understood.

We have constructed a 2-photon, 3-color detection Fluorescence Correlation Spectroscopy (FCS) microscope to measure the kinetic cooperativity in the assembling 30S ribosome. Up to three ribosomal proteins of interest can be labeled with different fluorophores, and the populations of up to seven different bound species with different combinations of labels can be discriminated by